The Standardised Mistletoe Extract PS76A2 Improves QoL in Patients with Breast Cancer Receiving Adjuvant CMF Chemotherapy: A Randomised, Placebo-controlled, Double-blind, Multicentre Clinical Trial

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Abstract. Patients with breast cancer receiving adjuvant chemotherapy frequently suffer from a restricted quality of life (QoL) due to the side-effects of chemotherapy and the consequences of coping with the diagnosis. Therefore, the objective of this clinical study was to investigate the impact of PS76A2, an aqueous mistletoe extract standardised to the galactoside-specific mistletoe lectin, on QoL by performing a placebo-controlled trial. Overall, 272 patients with breast cancer receiving adjuvant CMF chemotherapy (cyclophosphamide-methotrexate-fluorouracil) were enrolled and randomised to groups receiving placebo or PS76A2 at concentrations of 10, 30 or 70 ng mistletoe lectin (ML) per ml. The patients received 0.5 ml study medication twice weekly subcutaneously for 15 consecutive weeks (4 CMF cycles). Primary variables were the self-assessment QoL scores GLQ-8 (Global Life Quality) and Spitzer’s uniscale. As a result, statistically significant effects on QoL were obtained with the medium dose (15 ng ML/0.5 ml). The treatment difference between the medium dose and placebo with regard to the GLQ-8 sum was 60.8 mm (95 % confidence interval: 19.3 to 102.0 mm). The treatment effect for Spitzer’s uniscale between the medium dose and placebo was 16.4 mm (95 % confidence interval: 6.3 to 26.6 mm). The results on QoL were supported by an increase of T helper lymphocytes (CD4+) and the CD4+/CD8+ ratio (p<0.05). Overall, PS76A2 was well tolerated. Local reactions at the injection sites occurred dose-dependently, but were mild at the low and medium dose levels.

In conclusion, the medium dose of PS76A2 (15 ng ML/0.5 ml twice weekly) was shown to be safe and effective in improving QoL in breast cancer patients.

Aqueous extracts of the European mistletoe (Viscum album L.) have been widely used as a complementary therapy in cancer patients for decades (1). Although the pharmacological actions of mistletoe extracts or mistletoe lectins are well documented, data from clinical trials are still rare and the existing ones have been criticised as regards quality (2-5). Moreover, comparative review of the usefulness of mistletoe extracts in cancer therapy is severely hampered by the wide variety of commercially available mistletoe preparations which differ in terms of extraction and manufacture and are thus likely to display different pharmacological or clinical effects (6). The mistletoe lectins (ML) have been identified as the main active principle of mistletoe extracts (7). ML I has a broad range of affinity for galactopyranosyl residues. ML II is specific for D-galactose and N-acetyl-D-galactosamine, while ML III recognizes N-acetyl-D-galactosamine only. These heterodimeric glycoproteins belong to a group of type II ribosome-inactivating proteins (RIP) and are composed of a lectinic B subunit, which mediates cellular uptake of the disulfide-bonded hololectin and a cytotoxic A subunit, which inhibits protein biosynthesis enzymatically (8).

From experimental and clinical investigations, standardised mistletoe extracts and isolated ML have been demonstrated to possess immunomodulatory potencies by enhancing the secretion of cytokines and activity of immunological effector cells like natural killer cells and T lymphocytes. Moreover, inhibition of tumor cell proliferation has been shown in vitro and in numerous animal models (2, 9-13).

From these data, the question is whether therapy with mistletoe extracts has any significant impact on survival or the quality of life (QoL) of cancer patients. So far, no
Materials and Methods

Patients. A total of 272 female patients with operable breast cancer, who were eligible for adjuvant CMF chemotherapy, were enrolled in the study in 9 centres in Russia, Bulgaria and in the Ukraine prior to the start of the first cycle of CMF therapy. The main inclusion criteria were: TNM classification pT1-T3, N0-N+ (0-10 positive lymph nodes) M0, pre- and perimenopausal patients aged between 18 and 55 years and school education of more than 7 years. Exclusion criteria were: inability to answer the QoL scales, concomitant therapy with steroids (except on the day of i.v. chemotherapy) or biological response modifiers, concomitant therapy with steroids (except on the day of chemotherapy (17)).

The study was performed in accordance with GCP (Good Clinical Practice), the Declaration of Helsinki and the CONSORT statement. Prior to the start of the study, the approval of the ethics committees of the participating centres and the corresponding countries were obtained. Before enrolment, written informed consent was obtained from each patient.

Study design. The study was a randomised, placebo-controlled, dose-finding, double-blind, multicentre clinical trial with parallel treatments. Patients were allocated on the basis of a computer-generated randomisation list to the treatment groups [placebo and 10, 30 or 70 ng mistletoe lectin (ML)/ml]. The patients received 0.5 ml of the study medication PS76A2 (aqueous mistletoe extract standardised to ML; manufacturer MADAUSS AG, Cologne, Germany; commercially available as Lektinol®) subcutaneously twice weekly for 15 consecutive weeks. The study medications (placebo or verum) were identical in terms of appearance, smell, color and packaging. Each centre retained sealed emergency envelopes for each patient.

According to the protocol, patients had to fill in the validated QoL assessment scales GLQ-8 and Spitzer’s uniscale prior to any medical procedure. They were selected as primary efficacy variables because most of the known empirical clinical effects of mistletoe preparations are represented in both scales and, therefore, ensure a high sensitivity. Moreover, these scales are sensitive to changes in QoL during chemotherapy and are reliable validated instruments (14). The original English versions of the two scales were translated into Russian and Bulgarian according to a forward-backward translation. Oncologists fluent in both languages had to resolve discrepancies. Strict procedures for filling in the QoL scales had to be followed to reduce external influence factors like interaction with different personnel, locations, temperatures and timings (15, 16). The QoL scales were filled in at 6 visits: prior to each CMF cycle (during the controlled period patients received 4 cycles of CMF) and 2 and 3 weeks after the fourth CMF cycle covering an individual treatment period of 15 weeks.

On Days 1 and 8, the patients received the standard adjuvant CMF treatment consisting of 500 mg/m² cyclophosphamide, 40 mg/m² methotrexate and 600 mg/m² fluorouracil intravenously. During the controlled treatment period, patients received 4 cycles of CMF. To prevent or reduce nausea or emesis, patients received 10 mg dexamethasone i.v. and 10 mg metoclopramide q.i.d. per os on the day of chemotherapy (17).

As secondary variables, the QoL questionnaire QLC-C30 of EORTC, haematology, consumption of antiemetic and analgesic drugs, number of inpatient days, adverse effects and safety laboratory tests were included. Adverse events were recorded at each visit (days of CMF administration and 2 and 3 weeks after the fourth CMF cycle). Their causal relationship to the study drugs were assessed by the investigators under double-blind conditions. The immunological parameters determined in a subset of 43 patients were: natural killer (NK) cell activity and counts of NK cells, CD3+ HLA DR+ (activated T lymphocytes), CD4+ (T helper cells), CD8+ (suppressor/cytotoxic T cells), CD4+/CD8+ ratio, CD25+ (activated lymphocytes) as well as MAC1+ (macrophage-1 antigen).

Statistical analysis. An adaptive group sequential design with one interim analysis combined with a multiple testing procedure based on a priori ordered hypotheses, controlling the multiple α-level of 5%, was carried out (18-20). The interim analysis was intended to lead to either early termination in case of sufficient or missing treatment effects or continuation with a second independent trial step using the adaptively calculated sample size. The changes of the two primary variables (GLQ-8, Spitzer’s uniscale) from baseline were combined by means of O’Brien’s rank sum (21). Three a priori ordered tests were stipulated for this rank sum. (a) There is no trend between the treatments [contrast: -3 (placebo), -1 (low dose), +2 (medium dose), + 2 (high dose)]. (b) There is no trend between the three groups placebo, low and medium dose [contrast: -2 (placebo), +0.5 (low dose), + 1.5 (medium dose)]. (c) There is no difference between placebo and low dose [contrast: -1 (placebo), + 1 (low dose)].

A confirmatory two-stage analysis was to be conducted as follows: if the value from the first test was \( p_1 \leq 2.3\% \), the existence of a dose-response relationship was accepted and the study was stopped prematurely. The second null hypothesis was then to be tested using the test at the same significance level. In case of a significant result, the third null hypothesis was then to be tested using the test at an α-level of 2.3%, too. If \( p_2 \geq 50\% \), the existence of a dose-response relationship could not be shown and the study was to be stopped. If
2.3% ≤ \( p \) ≤ 50.0%, a second independent study step followed. The sample size was calculated adaptively and the final analysis was performed at the significance level of \( p \leq 0.0087 \). The choice of one-sided tests was justified because the aim of the study was to confirm the superiority of PS76A2 over placebo.

The sample size for the interim analysis was determined in the protocol under the following conditions: one-sided t-test situation, level of significance \( \alpha = 5\% \), power \( 1-\beta = 80\% \), standardised difference \( \Delta/s = 0.68 \). Therefore, it was necessary to enrol 36 patients per treatment group in the first step of the clinical trial. A total of 176 patients were randomised, because a drop-out rate of 20% was expected. For the immunological parameters, the non-parametric Jonckheere-Terpstra test for ordered alternatives was used.

### Results

**Patients.** The interim analysis was performed with 178 patients. The test for the first ordered hypothesis yielded a one-sided \( p = 0.0172 \) (≤0.023) for the combined endpoint. Therefore, a trend was confirmed and the four-arm study could be stopped according to Bauer and Köhne’s decision rules (18). The final analysis also included the patients recruited while the interim analysis was performed (overrunning). The results of the final analysis are presented here.

A total of 272 patients with stages II/III breast cancer without metastases, eligible for adjuvant CMF treatment, were enrolled into the clinical trial in 9 centres and allocated to randomised treatment. Of these patients, 261 completed the study and 11 patients were withdrawn prematurely due to adverse events (n=4), decision of the patient (n=4) or other reasons (n=3). All 261 completers (96%) were assessed in the intention-to-treat population (ITT). Overall, 18 patients with major protocol violations were excluded from the per protocol (PP) analysis, mostly (n=17) because the patients did not comply with scheduled visits. The results of the PP analysis did not differ from those of the ITT analysis.

The treatment groups were relatively well balanced with respect to demographics and medical history (Table I). However, the baseline values of the two primary variables were different between the treatment groups, but without reaching statistical significance [GLQ-8; \( p = 0.142 \), Spitzer’s uniscale (\( p = 0.109 \)] despite randomisation (Table II).

The confirmatory analysis of O’Brien’s rank sum of changes from baseline after 14 and 15 weeks (mean sum) in GLQ-8 sum and Spitzer’s uniscale revealed a significant trend (one-sided \( p = 0.0035 \), indicating a favourable global impact on

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### Table I. Demographical and anamnestic data.

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=70)</th>
<th>Low dose (n=67)</th>
<th>Medium dose (n=67)</th>
<th>High dose (n=68)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>Mean 43.5</td>
<td>Mean 45.5</td>
<td>Mean 44.6</td>
<td>Mean 45.5</td>
</tr>
<tr>
<td></td>
<td>SD 6.1</td>
<td>SD 5.3</td>
<td>SD 5.7</td>
<td>SD 5.5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>Mean 66.7</td>
<td>Mean 69.6</td>
<td>Mean 70.6</td>
<td>Mean 71.2</td>
</tr>
<tr>
<td></td>
<td>SD 10.5</td>
<td>SD 12.9</td>
<td>SD 11.2</td>
<td>SD 13.7</td>
</tr>
<tr>
<td>Breast surgery</td>
<td>radical /simple</td>
<td>65 (92.9) ↑</td>
<td>60 (89.6) ↓</td>
<td>59 (88.1) ↓</td>
</tr>
<tr>
<td>(mastectomy)</td>
<td>segmental</td>
<td>5 (7.1)</td>
<td>7 (10.4)</td>
<td>8 (11.9)</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>pN0 32 (45.7)</td>
<td>28 (41.8)</td>
<td>26 (38.8)</td>
<td>25 (36.8)</td>
</tr>
<tr>
<td></td>
<td>pN+ 38 (54.3)</td>
<td>39 (58.2)</td>
<td>41 (61.2)</td>
<td>43 (63.2)</td>
</tr>
<tr>
<td>Grading of</td>
<td>well-diff. 3</td>
<td>2 (3.0)</td>
<td>3 (4.5)</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>malignancy</td>
<td>moderate-diff. 33 (47.1)</td>
<td>32 (47.8)</td>
<td>32 (47.8)</td>
<td>35 (51.5)</td>
</tr>
<tr>
<td></td>
<td>poor-diff. 13 (18.6)</td>
<td>19 (28.4)</td>
<td>13 (19.4)</td>
<td>15 (22.1)</td>
</tr>
<tr>
<td></td>
<td>undiff. 5 (7.1)</td>
<td>3 (4.5)</td>
<td>5 (7.5)</td>
<td>4 (5.9)</td>
</tr>
<tr>
<td></td>
<td>not assessed 16 (22.9)</td>
<td>11 (16.4)</td>
<td>14 (20.9)</td>
<td>13 (19.1)</td>
</tr>
<tr>
<td>Oestrogen receptor</td>
<td>negative 12 (17.1)</td>
<td>7 (10.4)</td>
<td>12 (17.9)</td>
<td>7 (10.3)</td>
</tr>
<tr>
<td></td>
<td>positive 11 (15.7)</td>
<td>11 (15.7)</td>
<td>10 (14.3)</td>
<td>14 (20.6)</td>
</tr>
<tr>
<td></td>
<td>not assessed 47 (67.1)</td>
<td>49 (70.0)</td>
<td>45 (64.3)</td>
<td>47 (69.1)</td>
</tr>
<tr>
<td>Progesterone receptor</td>
<td>negative 12 (17.1)</td>
<td>8 (11.4)</td>
<td>10 (14.3)</td>
<td>2 (2.9)</td>
</tr>
<tr>
<td></td>
<td>positive 11 (15.7)</td>
<td>10 (14.3)</td>
<td>12 (17.9)</td>
<td>19 (27.9)</td>
</tr>
<tr>
<td></td>
<td>not assessed 47 (67.1)</td>
<td>49 (70.0)</td>
<td>45 (64.3)</td>
<td>47 (69.1)</td>
</tr>
</tbody>
</table>

↑Total number of patients (%)
QoL of the average effect of the medium and high dose of PS76A2 in comparison to the low dose and placebo. This result is comparable to the corresponding result of the interim analysis. Furthermore, a significant trend was shown (one-sided \( p = 0.0017 \)) in O'Brien's rank sum for the comparison of the medium and low dose compared to placebo. A clear superiority of the medium dose over placebo was obtained. The third test revealed no statistically significant differences between the low dose and placebo (one-sided \( p = 0.3532 \)).

Exploratory pair-wise comparisons between placebo and the active doses with regard to O'Brien's rank sum confirmed the superiority of the medium dose over placebo (two-sided \( p = 0.007 \), ITT). However, no superiority of the low dose (two-sided \( p = 0.7063 \)) and the high dose (two-sided \( p = 0.4003 \)) over placebo could be established.

The treatment difference between the medium dose and placebo with regard to the GLQ-8 sum was 60.8 mm (95% confidence interval: 19.3 to 102.0 mm). A tendency in favour of verum, especially the medium dose (Figure 1), was seen for each of the eight GLQ-8 items (Table II). Significant differences between the medium dose and placebo were obtained for tiredness, sexual interest or ability and thought of actually having treatment. Trends were seen for the items feeling sick (nausea and vomiting) and appetite or sense of taste.

In order to adjust the baseline imbalances between the four treatment groups with regard to the GLQ-8 sum, an analysis of covariance with the covariate baseline was additionally carried out. Marked differences between the treatment groups were seen at week 15 (placebo vs. medium dose: two-sided \( p = 0.0121 \), Figure 2). The baseline inhomogeneities in the items sexual interest and thought of actually having treatment did not lead to the superiority of the medium dose versus placebo regarding changes of GLQ-8.

<table>
<thead>
<tr>
<th>GLQ-8 sum [mm]</th>
<th>Placebo (n=66)</th>
<th>Low dose (n=66)</th>
<th>Medium dose (n=65)</th>
<th>High dose (n=64)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLQ-8 sum [mm]</td>
<td>128.9 (99.1)</td>
<td>157.2 (101.4)</td>
<td>171.5 (109.1)</td>
<td>158.5 (119.8)</td>
</tr>
<tr>
<td>1</td>
<td>35.7 (30.7)</td>
<td>38.1 (31.0)</td>
<td>41.5 (28.1)</td>
<td>39.8 (27.1)</td>
</tr>
<tr>
<td>2</td>
<td>11.9 (20.1)</td>
<td>15.1 (21.8)</td>
<td>17.4 (22.7)</td>
<td>13.4 (23.2)</td>
</tr>
<tr>
<td>3</td>
<td>14.0 (20.3)</td>
<td>14.4 (18.4)</td>
<td>15.7 (21.6)</td>
<td>17.4 (22.3)</td>
</tr>
<tr>
<td>4</td>
<td>3.7 (8.3)</td>
<td>7.0 (14.1)</td>
<td>6.0 (15.2)</td>
<td>8.1 (17.0)</td>
</tr>
<tr>
<td>GLQ-8 item 5 [mm]</td>
<td>31.2 (26.4)</td>
<td>31.0 (22.3)</td>
<td>36.0 (24.4)</td>
<td>35.3 (22.8)</td>
</tr>
<tr>
<td>6</td>
<td>11.2 (18.4)</td>
<td>13.2 (15.4)</td>
<td>16.4 (20.8)</td>
<td>12.5 (19.1)</td>
</tr>
<tr>
<td>7</td>
<td>12.8 (23.3)</td>
<td>27.1 (30.6)</td>
<td>21.8 (27.8)</td>
<td>20.7 (27.5)</td>
</tr>
<tr>
<td>8</td>
<td>8.3 (16.2)</td>
<td>11.3 (17.2)</td>
<td>16.7 (22.2)</td>
<td>11.2 (19.4)</td>
</tr>
<tr>
<td>Spitzer uniscale [mm]</td>
<td>35.1 (27.5)</td>
<td>39.5 (28.3)</td>
<td>46.4 (26.3)</td>
<td>37.9 (26.7)</td>
</tr>
</tbody>
</table>

The treatment effect between placebo and the medium dose with regard to Spitzer’s uniscale was 16.4 mm (95% confidence interval: 6.3 to 26.6 mm; two-sided \( p = 0.0016 \)).

The baseline adjusted course of Spitzer’s uniscale is presented in Figure 3. The treatment effect between the medium dose and placebo is significant after baseline adjustment (two-sided \( p = 0.0021 \)) as well.

No relevant differences between treatment groups were observed for the QoL scale QLQ-C30 of EORTC. Apart from immunological variables, no relevant differences were detected regarding changes from baseline after treatment for further secondary efficacy variables as haematological parameters, consumed numbers of paracetamol or metoclopramide tablets and inpatient days.

For immunological variables determined in a subset of 43 patients (Table III), a dose-dependent increase in cell number could be observed for T helper lymphocytes (CD4+) and the CD4+/CD8+ ratio after 4 and 15 weeks of treatment \( (p < 0.05) \). The NK cell activity was increased in the medium and more pronounced in the high dose group after 4 weeks; this effect was borderline significant \( (p = 0.05) \). Higher means of activated lymphocytes (CD25+) were seen for the medium and high dose group after 4 and 15 weeks of treatment. For the immunological variables CD4+ and CD4+/CD8+-ratio, a medium correlation with the GLQ-8 sum, which was tested significant, could be detected.

Safety. All 272 patients enrolled received at least one dose of study medication and were included in the safety population. More than 90% of the patients (n=262) received 4 cycles of adjuvant CMF chemotherapy during the controlled period. The mean number of study medication injections per patient were comparable in the four treatment groups.

Overall, 127 patients (46.7%) experienced a total of 244 adverse events. Adverse events occurring several times in a patient were counted as one adverse event (e.g. injection site
Figure 2. Baseline adjusted mean changes of GLQ-8 sum.

Figure 3. Baseline adjusted mean changes of Spitzer's uniscale.
reactions). For 4 patients (placebo: n=2, low dose: n=1, high dose: n=1), an adverse event led to the premature discontinuation of the trial. One patient of the high dose group stopped the intake of the trial medication due to an erythema, which was assessed as possibly drug-related by the investigator. After the withdrawal of the study medication, the erythema disappeared and the patient recovered completely. Several days later, the patient developed an ulcerous necrotic enterocolitis, a febrile neutropenia and an infectious toxic shock syndrome and died. The investigator and the pharmacological committee judged this serious adverse event as not related to the study medication.

The most frequent non-serious adverse event was a dose-dependent local reaction at the injection site (placebo: 0%; low dose: 9%, medium dose: 17.9%, high dose: 32.4%, p<0.001). The investigators assessed a possible or probable causal relationship to the study medication in all of these patients. Other probably or possibly drug-related adverse events (chill and muscle pain, allergic skin reaction, allergic conjunctivitis, headache) were observed in 4 patients (low dose: n=3, high dose: n=1).

As expected, in patients receiving chemotherapy white cell and reticulo-endothelial system disorders (placebo: 20%, low dose: 22.4%, medium dose 16.4%, high dose 20.6%) were the second most frequent adverse events. In addition, gastro-intestinal system disorders (placebo: 8.6%, low dose: 9.0%, medium dose: 9.0%, high dose: 14.7%), resistance mechanism disorders (placebo: 5.7%, low dose: 6.0%, medium dose: 7.8%, high dose: 8.8%) and red blood cell disorders (placebo: 5.7%, low dose: 3.0%, medium dose: 3.0%, high dose: 11.8%) occurred.

With the exception of local reactions at the injection sites, the other observed adverse events were common side-effects of chemotherapy with CMF and were not related to PS76A2 therapy. Furthermore, the analysis of the laboratory variables revealed no changes of clinical concern induced by the study medication.

There was a tendency for less leucocytopenia and granulocytopenia to be caused in the second part of the CMF cycles in the groups treated with PS76A2 compared to placebo.

Data quality assurance and audits. The following different types of audits were performed prior to, during and after conclusion of the study: a system audit of the Contract Research Organisation (CRO), which was responsible for the recruitment of investigators and monitoring of the trial, was performed by the Quality Assurance Department of the sponsor. The Standard Operating Procedures (SOPs) of the CRO, the personnel and the offices were checked and judged to be adequate for the conduct of the study according to GCP. Three on-site audits in Bulgaria and Ukraine of the study centres were performed by the Quality Assurance Department of the sponsor. The data quality regarding source data verification, accuracy and completeness was judged excellent for all centres. No relevant violations of GCP were reported.

Discussion

Patients with operable breast cancer (stages II and III) are generally treated with adjuvant chemotherapy after the complete removal of the tumor and axillar lymph nodes

Table III. Immunological variables at baseline and changes from baseline (%) after 4 and 15 weeks (w) of treatment [Means (SD)].

<table>
<thead>
<tr>
<th>Variables</th>
<th>Placebo (n=12)</th>
<th>Low dose (n=11)</th>
<th>Medium dose (n=11)</th>
<th>High dose (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ (%)</td>
<td>47.4 (7.5)</td>
<td>51.5 (2.9)</td>
<td>50.1 (7.3)</td>
<td>46.9 (5.3)</td>
</tr>
<tr>
<td></td>
<td>Change (4 w)*</td>
<td>-3.4 (15.8)</td>
<td>-1.6 (4.3)</td>
<td>1.0 (6.9)</td>
</tr>
<tr>
<td></td>
<td>Change (15 w)*</td>
<td>0.4 (10.3)</td>
<td>1.6 (6.4)</td>
<td>3.3 (8.5)</td>
</tr>
<tr>
<td>CD4+/CD8+</td>
<td>1.31 (0.30)</td>
<td>1.56 (0.39)</td>
<td>1.37 (0.37)</td>
<td>1.24 (0.34)</td>
</tr>
<tr>
<td></td>
<td>Change (4 w)*</td>
<td>-0.04 (0.24)</td>
<td>-0.1 (0.25)</td>
<td>0.14 (0.35)</td>
</tr>
<tr>
<td></td>
<td>Change (15 w)*</td>
<td>-0.12 (0.24)</td>
<td>-0.06 (0.18)</td>
<td>0.08 (0.43)</td>
</tr>
<tr>
<td>CD25+ (%)</td>
<td>34.8 (11.6)</td>
<td>31.9 (6.9)</td>
<td>32.9 (6.6)</td>
<td>36.6 (15.2)</td>
</tr>
<tr>
<td></td>
<td>Change (4 w)</td>
<td>-6.8 (15.0)</td>
<td>1.4 (7.6)</td>
<td>5.3 (7.4)</td>
</tr>
<tr>
<td></td>
<td>Change (15 w)</td>
<td>-4.0 (15.9)</td>
<td>1.5 (9.2)</td>
<td>3.2 (6.3)</td>
</tr>
<tr>
<td>NK activity (%)</td>
<td>14.9 (10.0)</td>
<td>15.4 (9.2)</td>
<td>13.1 (9.8)</td>
<td>14.2 (10.1)</td>
</tr>
<tr>
<td>Change (4 w)</td>
<td>-4.9 (7.7)</td>
<td>-5.5 (9.1)</td>
<td>-2.5 (8.6)</td>
<td>0.9 (8.5)</td>
</tr>
<tr>
<td>Change (15 w)</td>
<td>-8.8 (9.1)</td>
<td>-3.7 (12.4)</td>
<td>-3.8 (6.6)</td>
<td>-7.0 (6.3)</td>
</tr>
</tbody>
</table>

*Jonckheere-Terpstra trend test: p<0.05
(mastectomy or breast-conserving treatment) with the aim of prolonging the disease-free interval and improving overall survival. Patients are afraid of objective and subjective side-effects of chemotherapy. These side-effects and the patients’ ability to cope with diagnosis have a negative impact on the QoL and may lead to an avoidance of adequate administration of the chemotherapy, which results in a poorer prognosis (22, 23). The QoL of younger patients (<50 years) is more affected than that of older patients (24).

The results of this study demonstrated that PS76A2, a mistletoe extract preparation standardised to mistletoe lectin, improved the QoL of breast cancer patients receiving adjuvant CMF chemotherapy, as measured by GLQ-8 and Spitzer’s uniscale. The dose-response relationship indicated that the concentration of mistletoe lectin played a major role regarding the efficacy of the mistletoe extract preparation, which was also supported by experimental data (25).

The GLQ-8 sum and Spitzer’s uniscale are validated LASA scales for self assessment and proved to be sensitive to changes of QoL, which is supported by the work of other groups (23, 26, 27). However, for the secondary variable QLQ-C30 no relevant changes between treatment groups were observed. This is probably related to a lower sensitivity of this QoL scale, which represents a Likert scale. In another investigation, the global score of a Likert scale did not show any differences between healthy people and patients with breast cancer after treatment (28).

All 8 items of GLQ-8 showed differences between the medium dose and placebo, being largest for the items tiredness, sexual interest or ability and thought of actual having treatment. Marked differences in favor of the medium dose were seen for the items feeling sick (nausea and vomiting) and appetite or sense of taste. The effect on the item tiredness is of major importance for the QoL of patients with cancer, because more than three quarters develop fatigue during the course of their disease or treatment. Furthermore, approximately one-third of the patients reported that fatigue strongly affected their daily routines (29). Even after the end of the cancer treatment, patients continued to feel a profound tiredness that affected almost all aspects of life (30). From the patients’ point of view, tiredness, nausea, loss of hair and decreased sexual interest are major problems of chemotherapy (31).

In the verum groups, there were fewer adverse events which caused prolongations of CMF cycles than in the placebo group. The premature termination of chemotherapy and/or prolongation of intervals between cycles is related to a poorer outcome regarding disease- free interval and survival (32).

Apart from immunological variables, no relevant differences were detected regarding changes from baseline after treatment for secondary variables (haematology, consumption of paracetamol or metoclopramide tablets, number of inpatient days).

The observed influence on immunological variables may be of clinical relevance. CD4+ lymphocytes can eliminate cancer cells in vivo and play an important role in antitumor immunity (33, 34). The comparison of the treatment groups versus placebo revealed an increased natural killer cell (NK) activity during chemotherapy for the medium and more pronounced for the high dose group after 4 weeks of treatment. Increased NK activity levels may be of importance regarding a better clinical outcome of patients with cancer (35, 36). Higher levels of activated lymphocytes (CD25+) were seen for the medium and high dose groups after 4 and 15 weeks of treatment compared to placebo. Patients with operable breast cancer and higher levels of CD25+ and NK cells had a reduced recurrence rate compared to patients with lower levels (37).

The correlations found between immunological variables and improvement of GLQ-8 may form an explanation for the mechanism of action of PS76A2. The high dose was not as effective as the medium dose regarding GLQ-8, which might be explained by two reasons. Firstly, the increased rate of reactions at the injection sites in the high dose might have a negative impact on QoL and, secondly, the effect on QoL, which is probably mediated via the immune system, does not follow a linear dose-response relationship.

The compliance regarding administration of trial medication was excellent. None of the drop-out reasons was related to the study medication. In contrast to other published QoL data from cancer trials, the documentation and completeness of QoL data was very good in this study. This was achieved by intense monitoring, training of investigators and study personnel, as has been recommended by other groups (38).

The tolerability and safety of the trial medication was good. PS76A2-related local reactions at the injection sites did not lead to a discontinuation of treatment. Apart from this, no clinically relevant differences concerning adverse events and laboratory parameters between treatment groups and placebo were recorded. The observed adverse events, predominantly of the haematological and gastrointestinal system, are expected adverse events of the CMF chemotherapy and not related to treatment with PS76A2.

One patient of the high dose group died of an infectious toxic shock syndrome. The investigator and the pharmacological committee judged this serious adverse event as not related to the study medication. Adjuvant CMF chemotherapy is associated with a risk of mortality less than 1% (39). Pulmonary embolism is the most common cause of death. Several deaths attributed to sepsis or toxic effects have been related to CMF treatment (40).

This study demonstrated that the medium dose of PS76A2 (15 ng ML/0.5 ml twice weekly) improved the QoL of patients with breast cancer receiving adjuvant CMF chemotherapy. Taking into consideration that the adjusted GLQ-8 baseline levels were 154 mm and the mean...
improvement of GLQ-8 was 61 mm of the medium dose compared to placebo, the observed effects (40% improvement) were clinically relevant. Clinical studies with other mistletoe extract preparations in patients with advanced breast or colorectal cancer and a drug monitoring study on patients with different malignancies support the results of this study (41-44). The obtained dose-response relationship provides the rationale for using preferably standardised mistletoe preparations like PS76A2 in future clinical trials.

In conclusion, the standardised mistletoe extract preparation PS76A2 was shown to be safe and effective in improving the QoL in breast cancer patients at a dose of 15 ng ML/0.5 ml twice weekly.

Acknowledgements
We would like to thank all the investigators for participating in this clinical trial: Prof. Chernoysmij, Prof. Tetzschke (Sofia, Bulgaria), Prof. Kozhauer, Dr. Ozhinnikova (Moscow, Russia), Dr. Tarutinow, Dr. Yaremchuk (Kiev) and Prof. Bilinsky (Lviv, Ukraine). S & P Pharmatech GmbH (Berlin, Germany) organised the monitoring of this study. The Institut für Angewandte Statistik (Bielefeld, Germany) carried out the data management and the statistical analysis. Mr. M. Bulitta (CRM Biometrics GmbH (Rheinbach, Germany) was involved in the statistical analysis and the writing of this paper. X-act (Cologne, Germany) provided the randomisation list and the emergency envelopes. Covance (Basel, Switzerland) was responsible for packing and distribution of the study medication. The Laboratory of Clinical Immunology, University Hospital St. Ivan Rilski (Sofia, Bulgaria) carried out the immunological measurements. MADAUS AG (Cologne, Germany) provided funding for this study and the study medication. Finally, we thank Prof. Uberla (Munich, Germany) for his valuable advice.

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